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Comparison of slit sampler performance in a biological aerosol chamber

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Abstract

The history on slit to agar samplers shows that the first published description was on a system using 10 cm petri plates. At some point after the 1950's samplers that collected biological aerosol particles on 15 cm plates appeared. More recent patents have been filed that described slit samplers using 10 cm plates, usually citing economics as the prime motivation. Yet workers have cautioned that the smaller plates can become saturated when heavy aerosol clouds are encountered. In applications where the slit sampler is used as reference collector when biological detectors are under test, heavy clouds may often be encountered. This report will demonstrate how to determine performance characteristics of slit samplers designed for either 10 or 15 cm plates. It will also show the importance of controlled replicate measurements providing data suitable for rigorous statistical analysis. The results indicate that for measuring biological clouds of between 5 to 30 agent containing particles per liter of air (ACPLA) the 15 cm plate sampler design is more efficient than those targeted for the smaller collection surface. A statistical method has been employed to test difference between regression slopes.

Résumé

L'histoire sur la fente aux échantillonneurs d'agar prouve que la première description éditée était sur un système à l'aide des plats de 10 centimètres Pétri. À un certain point après les échantillonneurs 1950.s que les particules biologiques rassemblées d'aérosol des plats de 15 centimètres sont apparues. On a classé des brevets plus récents qui ont décrit des échantillonneurs de fente à l'aide des plats de 10 centimètres, habituellement citant des sciences économiques comme motivation principale. Pourtant les ouvriers ont averti que les plats plus petits peuvent devenir saturés quand des nuages lourds d'aérosol sont produits. Dans les applications où l'échantillonneur de fente est utilisé comme collecteur de référence quand les détecteurs biologiques sont à l'essai, des nuages lourds peuvent souvent être produits. Cet état démontrera comment déterminer des caractéristiques d'exécution des échantillonneurs fendus conçus pour des plats de 10 ou 15 centimètres. Il montrera également que l'importance de commandé replient des mesures fournissant des données appropriées pour l'analyse statistique rigoureuse. Les résultats indiquent que pour mesurer les nuages biologiques entre de l'agent 5 à 30 contenant des particules par litre d'air (ACPLA) la conception d'échantillonneur de plat de 15 centimètres est plus efficace à ceux visées pour la surface plus petite de collection. Une méthode statistique a été utilisée pour tester la différence entre les pentes de régression.

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Executive summary

The only practical way to measure live biological particles in air is to collect the aerosol particles via impaction on growth medium and then incubating the plates overnight to detect colony formation. With these results, agent containing particles per liter air (ACPLA) can be calculated. The term ACPLA is the critical unit of measure for biological aerosols accepted by all workers in the detection field. The slit sampler is a rugged instrument that can perform this task. We have examined the performance characteristics of different slit sampler designs, primarily ones with different slit length and impaction surface area. Having a short slit length design allows the use of smaller diameter petri plates for low cost particle collection. Although this may have economic advantages, there are definite compromises in terms of performances. Our studies have demonstrated a relationship between slit length and regression slope, the latter term defined as a measure of sampling efficiency. We have also introduced a method to objectively compare regression slopes that has provided to be a convenient way to grade sampler performance. We recommend that future work with slit samplers should include verification that data collection has not been distorted by colony overlap errors. Concern has been raised that sampling errors may occur while using the slit sampler in horizontal laminar air flow as in a wind tunnel. Fortunately, in field applications, horizontal laminar flow conditions were rarely encountered in nature. We submit that colony overlap errors may pose more significant problems when working in natural settings where biological content density can be difficult to predict. Finally, we have defined the concept of transfer functions as a way to correct for slit sampler design deficiencies, to be used for correcting either historical or current data.

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Sommaire

La seule voie de mesurer les agents de phase en air est de rassembler les particules d'aérosol par l'intermédiaire de l'impaction sur le support de croissance et les plats incubant durant la nuit pour détecter la formation de colonie. Avec les résultats, l'agent contenant des particules par air de litre (ACPLA) peut être calculé. La limite ACPLA est l'unité de mesure critique pour les aérosols biologiques reçus par tous les ouvriers dans le domaine de détection. L'échantillonneur de fente est un instrument raboteux qui peut accomplir cette tâche. Nous avons examiné les caractéristiques d'exécution des différentes conceptions d'échantillonneur de fente, principalement ceux avec la longueur différente de fente et la superficie d'impaction. Avoir une conception courte de longueur de fente permet l'utilisation de plus petits plats de Pétri de diamètre pour la collection de particules à prix réduit. Bien que ceci puisse avoir des avantages économiques, il y a des compromis définis en termes d'exécutions. Nous avons démontré un rapport entre la longueur fendue et la pente de régression, le dernier précédemment définies comme mesure d'efficacité de prélèvement. Nous avons également présenté une méthode pour comparer objectivement des pentes de régression, fournissant une voie commode d'évaluer l'exécution d'échantillonneur. Nous recommandons que les travaux futurs avec les échantillonneurs fendus devraient inclure la vérification que la collecte de données n'a pas été tordue par des erreurs de superposition de colonie. Le souci a été augmenter que les erreurs de prélèvement peuvent se produire tout en en utilisant l'échantillonneur de fente dans la circulation d'air laminaire horizontale aiment dans un tunnel de vent. Heureusement, dans l'application de zone, des états laminaires horizontaux d'écoulement ont été rarement produits en nature. Nous soumettons avec respect que les erreurs de superposition de colonie peuvent poser des problèmes plus significatifs en travaillant dans les configurations normales où il peut être difficile prévoir densité contente biologique. En conclusion, nous avons défini le concept des fonctions de transfert comme voie de corriger pour des insuffisances fendues de conception d'échantillonneur, pour être utilisé pour tordre des données historiques ou actuelles.

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Acknowledgements

Participants came from great distances to make this work possible. We thank S. Farrow and J. Burke for setting up the different slit sampler designs in performing these studies.

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Introduction

Due to recent interest in environmental and indoor air quality, slit samplers have been given more attention from workers in aerobiology (Jericho et al, 2000, Anderson et al, 1996). In the past decade, two separate patents for slight variations of the slit sampler have been awarded (Kiplinger, 1996) the latest as recently as in 1998 (Swenson, 1998). However, very rarely are slit samplers the sole instrument of choice for viable biological measurements even though its effectiveness has been recommended by a number of workers (Morris et al 2000). More commonly, users deploy a variety of samplers of varying designs, probably as a hedge. Casewell et al (1984) compared the Reuter centrifugal air sampler (RCS) with the Casella slit sampler using *Bacillus globigii* spore aerosol. Their results showed that the slit sampler gave counts that were apparently three to tenfold higher than those with the centrifugal sampler. A few years later, the same authors performed more measurements of hospital air and concluded that the RCS could replace the slit sampler primarily due to its convenience of use and portability (Casewell et al, 1986). Given the diversity of sampler choices, Henningson and Ahlberg (1994) did a very comprehensive review of biological aerosol samplers and in their paper, slit samplers were given good coverage.

Comparative studies on slit samplers appear to be a fairly common scientific activity. Groschel (1980) reported that the Ross Microban sieve sampler and the Biotest Reuter Centrifugal Sampler when tested with the Casella slit sampler, showed comparable results. In fungal studies, Smid et al (1989) reported on the sampling efficiencies of the Andersen N-6 sampler, slit sampler and Reuter centrifugal air sampler (RCS), concluded that the N-6 and slit sampler performed best. Observations similar to this was confirmed by Verhoeff et al (1990) who performed studies with the same instruments. In contrast, when Placencia et al (1982) measured bacterial recovery by a portable Reuter centrifugal air sampler and a standard Mattson-Garvin slit-to-agar air sampler, they found the former yielded significantly higher recoveries.

Heeg and Kanz (1975) using a Casella slit sampler, placed in the middle of a intensive care unit, established that human activities caused bacterial count to rise by 35-310 percent above the control level. When the dust content of the air was measured with a Royco particle counter, it showed close correlation between changes in the number of particles (>5 μm) with respect to the number of airborne bacteria. These examples serve to illustrate that there is no clear literature consensus for the best sampler when the experimenter needs to collect viable particle information. Clearly, there is a need for a method that permits replicate measurements as well as a statistical method to objectively evaluate results.

The slit sampler was first described by Bourdillon et al (1941) over 60 years ago. Since then it has been used as a baseline reference for other types of samplers (Lach 1985). Commercial versions (Biap Slit-Sampler) have been used for various environmental applications like fungal measurements in Copenhagen (Larsen 1981) and bacterial contaminations in slaughterhouses (New Brunswick STA 200, Jericho et al, 2000). Tjade and Gabor (1980) mentioned that the Biap slit sampler and the Casella Mk 2 slit sampler when studied in an orthopaedic operating theatre showed similar bacterial counts in the range of 74-640 cfu. (colony forming units)/m^3 air. Most of these samplers have timing clocks that revolve at 1 or 2 revolutions per hour. In a previous report we describe a high resolution slit sampler that was a modification of the New Brunswick Model STA 200. The sampler was capable in resolving a three second time interval of a passing aerosol cloud (Ho et al. 1999). This paper

describes a comparison between this sampler to a Mattson-Garvin slit-to-agar sampler that also has high resolution modifications done to it. In addition, the comparison study also included a sampler that used a 10 cm plate as particle collection medium. An aerosol chamber has been used to perform reproducible experiments. We also introduce a statistical approach to objectively compare the performance of slit samplers that have been run under these reproducible experimental conditions.

Methods and materials

Aerosol chamber

Biological aerosol dissemination (spores of *Bacillus subtilis* var *niger*, ATCC 9372, BG, also called *Bacillus globigii*) was accomplished with two Hudson nebulizers (model 1700, Hudson Oxygen Therapy Sales Co.,Wadsworth, OH) at 172-206 KiloPascal (25-30 psi). Fans were used to keep the aerosol particles well mixed. A suspension containing BG in the range of 5 to 30 µg plus 4 mg silica gel (Syloid 245, Davison Chemical, Baltimore, MD) per ml was used as the starting material. For each experiment, the dry BG powder was weighed out from a stock batch. For example, to prepare a 10 µg/ml suspension, 0.1 gm dry powder was weighed out (model AE 200-S, Mettler Instruments, Zurich, Switzerland) and added to 10 ml distilled water. Serial 1:10 dilutions were repeated 3 times to obtain a final concentration of 10 µg/ml. A working volume of 25 ml was used to fill the aerosol generator. Actual viable counts in the suspension were determined to obtain the source concentration.

A steady aerosol concentration in a 45 m^3 chamber was achieved via a feed back control loop mechanism as previously described (Ho, 1989). In this scheme, a particle sizer was used to measure aerosol concentration at every 5 second interval. The result was compared to a previously defined threshold; custom software performed the control function to regulate switching of the aerosol generator. The monitored particle size range was 0.7-1.0 µm at 1 particle per cc concentration threshold. Steady state aerosol concentration was achieved in about 15 minutes from startup. Syloid was used to provide sufficient particle counts in the aerosol to maintain efficient operation of this feedback loop. Using µg/ml quantities of BG without the presence of Syloid could not generate sufficient particles to perform the feed back looping operation.

Sampler collection

Critical to understanding the characteristics of the test aerosol, culturable particles in air must be captured and grown to determine the presence of "live" content. To properly characterize the BG particulate material, a number of standard biological aerosol collectors were used (Chatigny et al. 1989). Biological aerosol particles were impacted onto 15 cm nutrient agar plate situated in a slit sampler (model STA 203, New Brunswick Scientific, Edison, NJ 08818-4005). The air flow rate was 50 liter/min. Each sampler was electronically modified to rotate at 1 revolution/min. A sampler array consisted of 10 devices serially connected to function as a continuous 10 minute collector. After overnight incubation, live particles were counted as bacterial colonies by means of a flat bed scanner driven by custom software developed jointly by DRES, Dugway Proving Ground (DPG) and Spiral Biotech (model "STAR" version 1.5, Spiral Biotech, Bethesda, MD). Slit sampler data were expressed as agent containing particles per liter of air (ACPLA).

A dichotomous sampler, DS, (model 245, Andersen Samplers Inc., Atlanta, GA) operating at 17 L/min, collected particles on a set of 2 glass fiber filters representing fine and coarse fractions (Ho, 1991). Sampling was done in 10 minute batches, repeated three times. The filters were disrupted in 20 ml water using a wrist action shaker (model 75, Burrel corp. Pittsburgh, PA). Wire gauze disks were used to clarify the supernatant before viable spore assessment.

Viable spore assay

Viable organism enumeration was by spiral plating technique as described by Hedges et al. (1978). Liquid samples were applied to standard nutrient agar plates with a spiral platter (model 4000, Spiral Biotech Inc. Bethesda, MD). The plates were incubated overnight at 37° C and analysed with the CASBA 4 System that combines a high-resolution line scanner and specialised Windows® based software to count microbial colonies on agar plates. Custom software developed by DRES and DPG were used to analyse slit plates. The Colony Image Analysis (CIA) software locates and analyses each colony and provides the plate and sample count. Data output in Excel format can be further processed by conventional software. Statistical analysis was done with SigmaStat version 2 (SPSS Inc. 444 N. Michigan Avenue, Chicago, Illinois, 60611).

Slit Sampler design to achieve high resolution capabilities

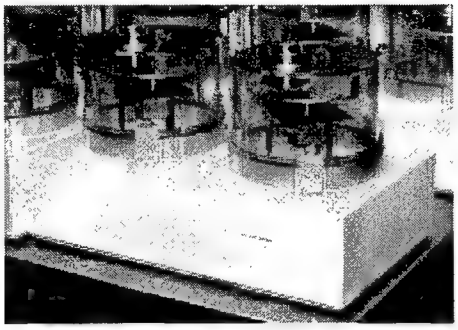
We consider convention slit samplers that revolve at 1 to 2 revolutions per hour with air flow rate of 50 liter/min as low resolution instruments. They are good for normal natural environment measurement where few live particles are known to exist. In contrast, high resolution sampling is defined as the ability to resolve aerosol cloud concentrations in 1 to 3 second time slices with the petri plate platform revolving at 1 to 2 revolutions per minute. High resolution biological aerosol sample collection is not a new concept. Since the 1950's slit samplers that ran at one revolution per minute have been commercially available (Model FD 100, Reyniers & Son, Chicago, IL) but these were driven by mechanical clock works and were not suitable for serial sampling requirements. Serial sampling is a simple concept that describes a set of electronically networked instruments each with an address that can be activated in sequence or any combination thereof.

High resolution sampling was made possible from a DRES-DPG collaborative effort by which the 15 cm petri plate scanner was developed (Ho et al. 1999). The hardware and software system permitted automated data reduction which was essential for field trials that generate many sample plates that had to be processed within a short time, a restriction due to microbial growth characteristics. In the first iteration, stock New Brunswick slit samplers were modified by a replacement digital motor together with the appropriate driver board interface and serial switching and timing circuitry. This work was done by a contractor (Dycor, Edmonton Ca.) and the instrument was called the Dycor modified New Brunswick. Unfortunately, the original slit sampler platform went out of production so recent attempts to acquire more units required radical strategic changes to be made.

Fortunately, key components such as the original slit cup (slit dimension 0.15 x 48 mm) and the plastic chamber bowl could be obtained from New Brunswick as spare parts. With these items, it was possible to construct a sampler that should theoretically perform like the original. While redesigning this unit, it was decided that by putting two slit and bowl units on one base, operational efficiencies could be realized (figure 1). The integration and electronics for this dual slit platform was performed by another contractor (HF Research, Medicine Hat, Alberta) and thus the instrument was called the HF modified slit system. The only major specification deviation from the standard slit sampler was that the flow rate was changed to 33 liter/min due to the solenoid valve flow restriction.

Dycor modified New Brunswick

HF modified



10 cm Plate design

Mattson-Gavin Model 220

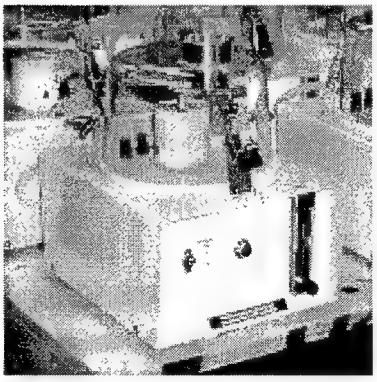


Figure 1. ***Instruments used in aerosol chamber measurement studies.***

As mentioned in the Placencia et al (1982) paper, Mattson-Garvin offers a model 220 slit sampler (Barramundi Corporation, Homosassa Springs, FL) with interchangeable drive motors (60, 30, 15, 5 minute). Their web site describes the instrument as "The design philosophy of the M/G Sampler is based upon drawing air through a 0.152 mm slit at 1-cubic foot/minute and impinging the particles contained therein upon an agar surface 2-3mm below the slit. The agar is contained in a commercially available 150 mm x 15mm disposable culture plate which is rotated by a synchronous drive motor. The rate of rotation can be varied by the interchangeable drive motors. After sampling, the plate is incubated and the colonies counted. This count reflects the number of airborne particles carrying viable organisms. No dilution or plating steps are required. Results are expressed as viable particles per unit of air and a time-concentration relationship may be determined." (http://www.mattson-garvin.com/models.htm). In the present comparison, a MG sampler with a two minute per revolution motor drive was used.

Bourdillon et al (1941) originally designed their slit dimension at 0.25 by 27.5 mm for a standard 10 cm petri plate. In contrast, the Casella sampler, also using a 10 cm plate, mentioned by Groschel (1980), had a very similar set of slit specifications (0.3 x 28 mm). Historically, it is possible that Fort Detrick (1959) could have evolved the slit design to current 0.15 x 48 mm. For this series of comparison measurements, a 10 cm plate design (Solectron Ltd, Fareham, Hampshire, UK) using a 0.3 x 17.5 mm slit was also tested.

In the current experiments, the following sampling systems were located in the chamber and operated simultaneously: 1. Mattson-Gavin, 2. Dycor modified design, 3. HF modified design and 4. the 10 cm design operating at flow rates shown in Table 1.

Table 1. Flow rate (LPM) characteristics for the 10 cm design and the Dycor modified New Brunswick. The conversion factors were used to estimate ACPLA values for the 10 cm design, taking into account the different flow rates. For the other instruments, flow rates and conversion factors used for calculation of ACPLA were as follows: HF = 33 (1/1.1); Mattson-Garvin = 28.3 (1/1.89). Even though each Dycor modified sampler had different flow rates, for calculation purposes, 50 LPM was used (see text for explanation).

Sampler Index	10 cm design	Conv. 1/x	Dycor Modified NB
1	16	1.07	41
2	14	0.93	22
3	8	0.53	34
4	12	0.8	42
5	12	0.8	37
6	18	1.2	29
7	18	1.2	42
8	11	0.73	24
9			40
10			44

Statistical analysis

TableCurve 2D was used to derive slope and linear regression statistical calculations (Table Curve version 4, SPSS, Chicago, IL). The program produces a graphical plot with all the relevant statistical values. Comparison of slopes was done according to the method described by Armitage and Berry (1995). The routines were rendered in an Excel spreadsheet and tested by using the sample data provided in the book.

Results and discussions

Aerosol measurements, unlike analytical chemistry determinations, are subject to great numerical variances influenced by a variety of parameters that are difficult to control. Slight variations in experimental maneuvers can contribute to different results that may appear unacceptable. For this series of chamber experiments, we structured an approach that would account for variations such as the act of weighing the starting material. It can be seen that overall, larger amounts of dry BG material, when rendered into suspension, gave correspondingly greater viable counts in each replicate experiment. When the mean viable counts of the replicate are compared, Figure 2 shows that there is a log-linear relationship with respect to dry weight.

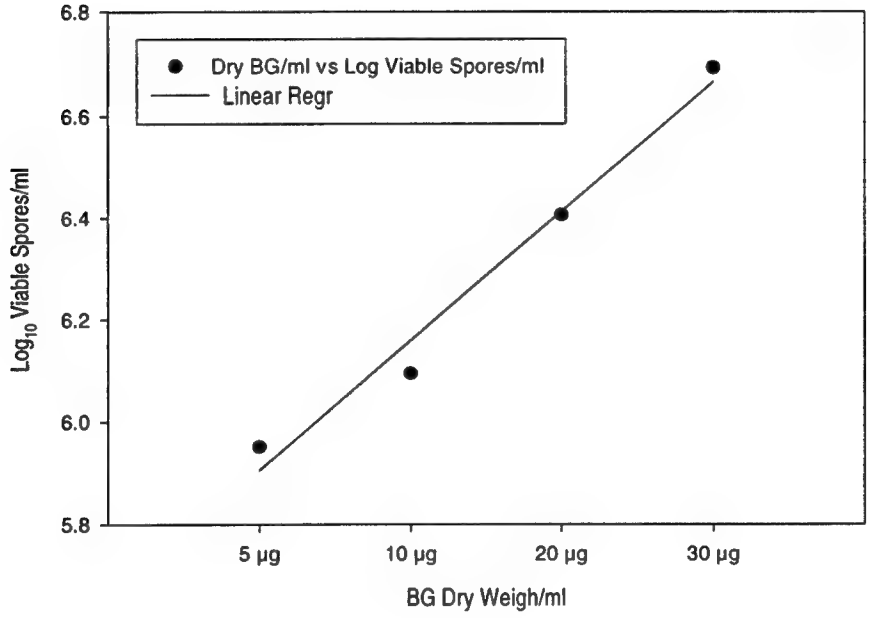


Figure 2. ***Plot of dry BG powder by weight vs mean log viable spores in one ml of liquid suspension. Each experimental run starts with the weighing of dry BG powder to take into account weighing errors that may affect final outcome. In practice, 50 to 100 mg batches of the material were deemed appropriate for weighing accuracy. Working suspensions were serially diluted to the final concentrations that are used in the sprayer. Viable spore counts were performed on the final preparation.***

Before each experimental run, conventional low resolution slit samples were used to determine if there was any background aerosol particle contamination. As shown in Figure 3, the chamber was very clean except for when a worker went into the space.

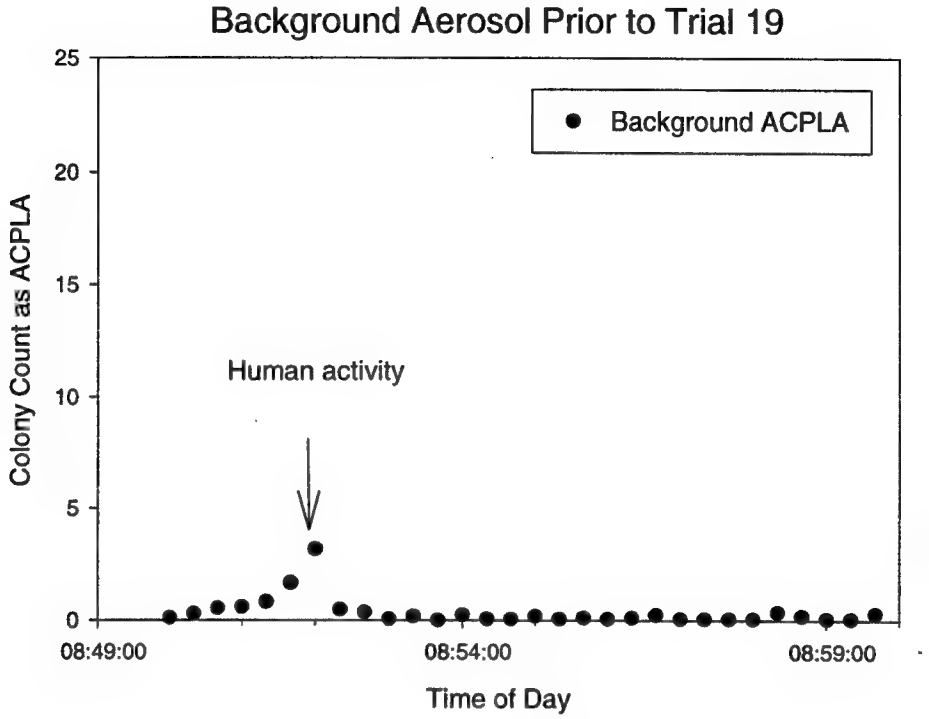


Figure 3. ***Typical background aerosol concentration in the chamber before the start of an experiment. Note the slit samplers are capable of registering about 4 ACPLA, the presence of contaminants primarily due to the human activity stirring up residual material on chamber surfaces.***

Aerosol characterization with slit samplers.

After about 15 minutes from aerosol generator startup, equilibration of the aerosol concentration was achieved, as determined by on/off cycling of the software controlled system. Slit sampling sequence was initiated via remote control that activated the first in a series of 10 devices. Concomitantly, the vacuum source was also activated providing the desired air flow for each of the systems. With each sample plate revolving at the stated rate each provided a very fine resolution of the instantaneous aerosol concentration encountered during the 20 minute experiment. When the resultant colony counts were analyzed for each of the 30 segments per plate, each representing a 4 second time slice, the 300 data points can be

depicted first as a scatter plot (Figure 4) and then as a frequency distribution plot as shown in Figure 5. Each data set (n = 300) for all the sample designs when subjected to normal

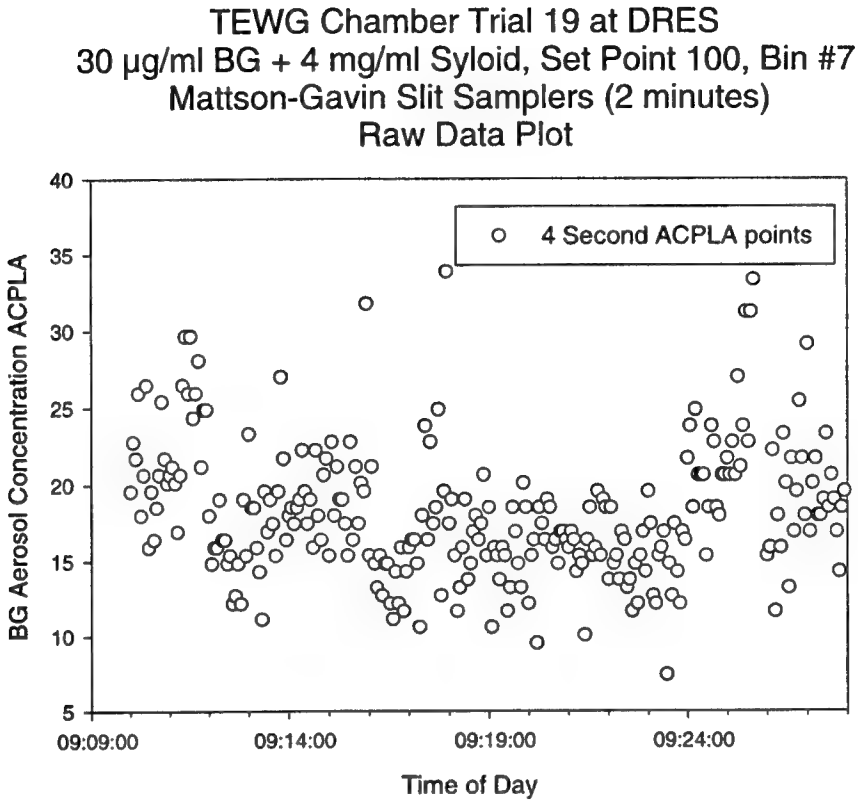


Figure 4. ***Typical high resolution profile of the viable particle concentration measured as 4 second time slices. The whole experimental run was performed in 20 minutes using an array consisting of 10 slit samplers each sequentially sampling at 2 minutes each. During colony counting, each plate was divided into 30 sectors each representing 4 seconds of particle impingement on the agar surface. The operational flow rate (28.3 LPM for Mattson-Garvin design) and the colony counts was used to determine ACPLA.***

distribution testing, failed without exception. This confirms similar observation for field trial measurements of biological aerosol using optical methods (Ho et al. 1999). Tillett and Carpenter (1991) also reported that raw epidemiological data in microbiology were not normally distributed, thus recommending the use of non-parametric statistical techniques.

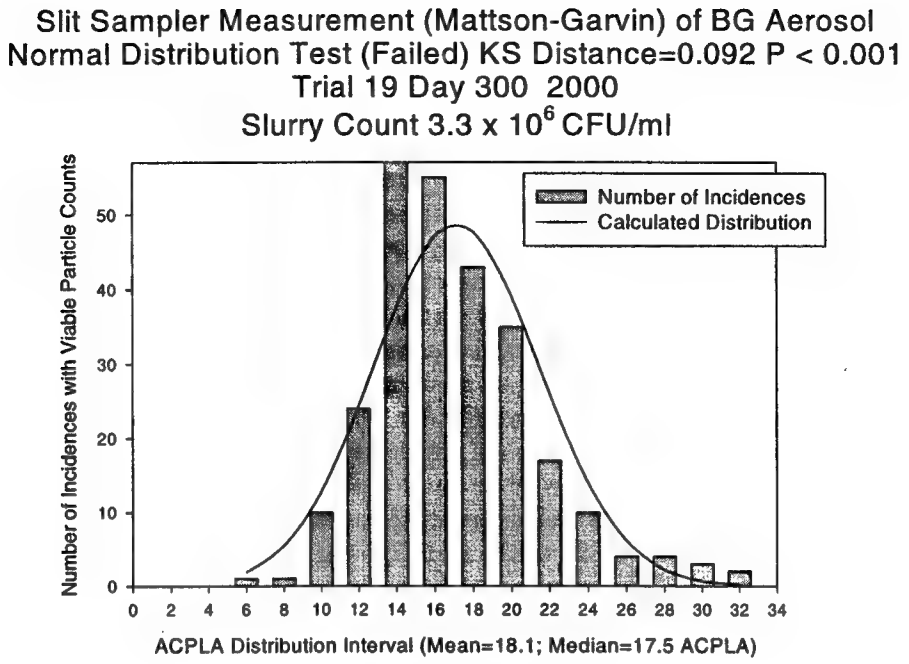


Figure 5. ***Typical viable count frequency distribution showing non-normal characteristics. This is an essential basic test for all microbiological data to determine if non-parametric statistical analysis techniques are required. The median rather than the mean is an appropriate description. Each experimental run produced a median value that described the overall concentration profile that was estimated by the slit sampler design.***

Instead of obtaining the mean over the 20 minute sampling period, the appropriate way is to report the median aerosol concentration, expressed in agent containing particles per liter of air (ACPLA). In Figure 5, the median (17.5) was mathematically derived by the software that performed the distribution calculation. Numerically this was slightly lower than the estimated mean (18.1). For consistency and statistical correctness, all subsequent slit sampler data are reported as median ACPLA, obtained over the sampling period.

Figure 5 gives a very good illustration of how biological aerosol sampling is best described as a population study where at any given time, the actual concentration can be anywhere between two extremes of the distribution. In this example, there were samples that registered in the low end at 6 while others were in the high at 32 ACPLA. To fully describe biological aerosol characteristics, we recommend that these descriptive treatments should represent minimal requirements.

Each experimental run, using a known concentration of spore suspension, provides one data point that is a statistical representation of the viable particle concentration, expressed as the median ACPLA. When the suspension strength was changed, one would expect the median ACPLA to behave correspondingly and the results are summarized in Figure 6. Given the complexity of the data handling process, we were gratified to see that median ACPLA values appear to increase with increasing suspension concentration. Moreover, when the data points were fitted to a linear regression routine, a respectable coefficient of 0.938 was obtained for the Mattson-Garvin array. Due to the clean background conditions in all experiments (Figure 3) we have mathematically forced the extrapolated curve to pass through the origin. Another observation was that most of these points were within the 95% prediction boundary and that the transformed data plot was normally distributed. According, as shown in Figure 6, conventional parametric statistical descriptions could be used to characterize the data plot. By inspecting the curve, it can be seen that a number of experiments yielded data points close to the 5 ACPLA mark, suggesting that it may be possible to repeatedly establish such low biological aerosol concentration in the chamber. Indeed, Figure 6 provides useful information as the starting point to permit the user to select a desired ACPLA level for detector challenge work. By picking an appropriate suspension concentration, there is reasonable probability that the desire median APCLA can be obtained.

Performance by other samplers

The results for the other sampler systems are shown in Figure 7 (Dycor modified), Figure 8 (HF modified) and Figure 9 (10 cm design). As mentioned before, the Dycor modified samplers had varying flow rates (table 1) but that fact was not known when the calculations were done to produce the plot in Figure 7. We decided to show the plot unaltered to demonstrate the results of a poorly maintained sampler system. It was rather surprising to note that the composite results did not appear too out of line when compared to the Mattson-Garvin instrument. In particular, Figure 7 revealed that the Dycor instrument array passed both the normality and variance test. Both the regression coefficient and the standard error were fairly acceptable. The only poor mark was the slope that appeared lower when compared to the HF design.

It was difficult to predict the performance characteristics of a slit sampler designed for a smaller particle capture surface as in the 10 cm plate design when compared to the large surface. One positive observation seen in Figure 9 was that the regression coefficient was very good (0.918) and that the standard error was very low (0.588). However, when comparing the linear regression slopes of all the systems (Figure10), the 10 cm design sampler rated at the bottom.

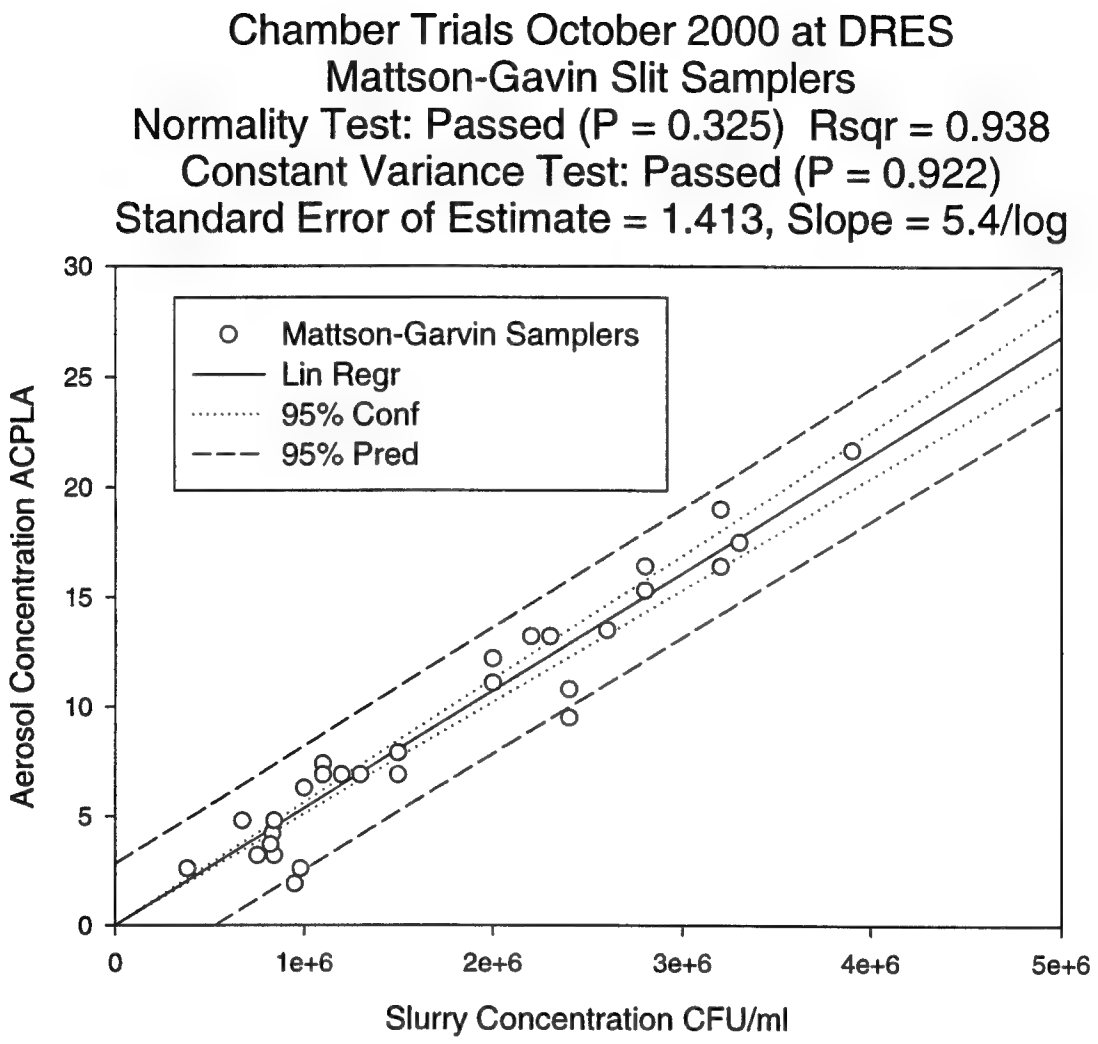


Figure 6. ***Typical data plot profile of median values obtained from different experimental runs. Each run was started using a different spore suspension concentration, thus resulting in varying aerosol concentration as shown in this figure. The pooled data when fitted to a linear regression prediction gave normally distributed relationship. This plot demonstrates the results from the Mattson-Garvin sampler.***

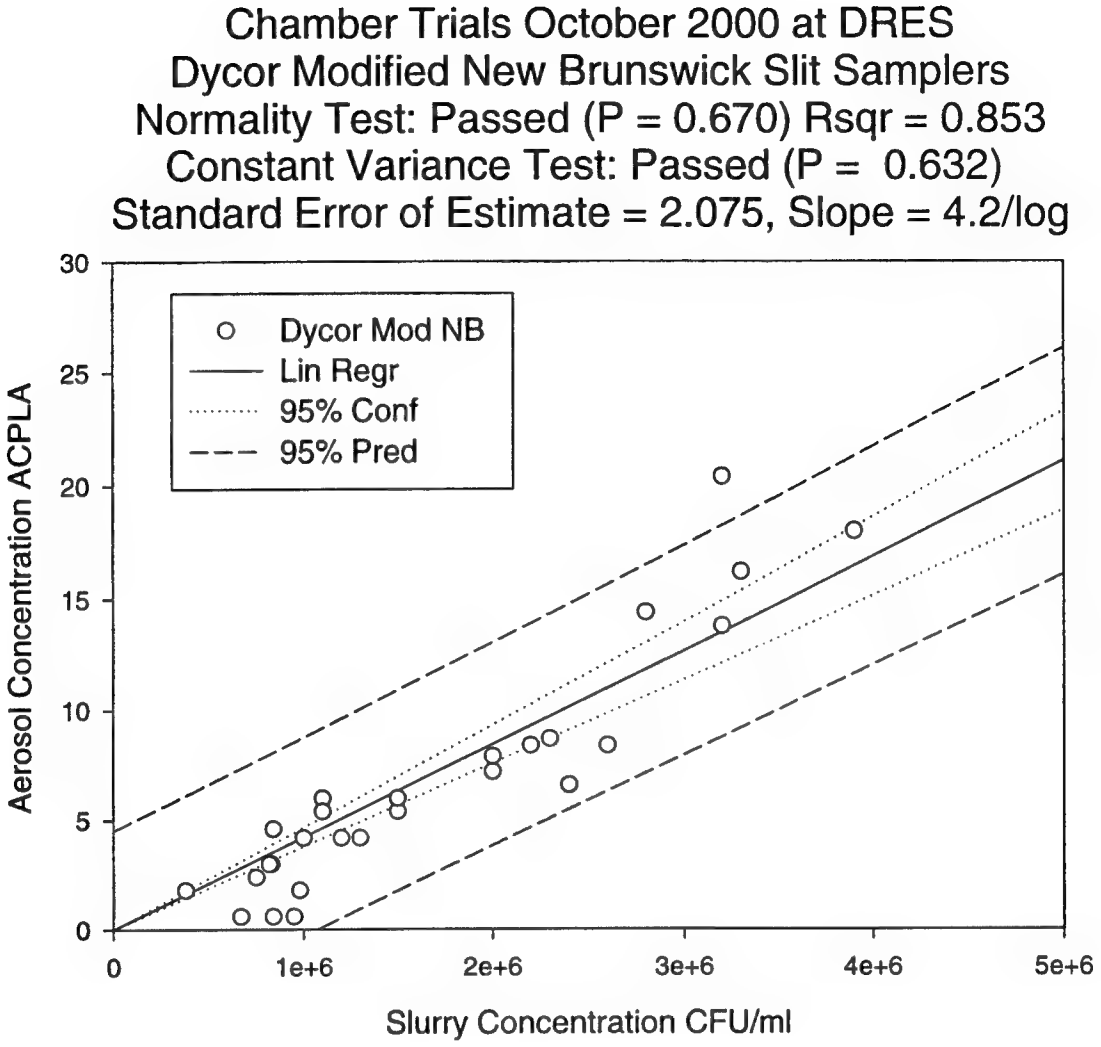


Figure 7. ***Plot profile of the Dycor modified slit sampler. In table 1, it was noted that although 50 LPM was used for calculating the ACPLA values, the actual flow rates varied widely due to dirty slits.***

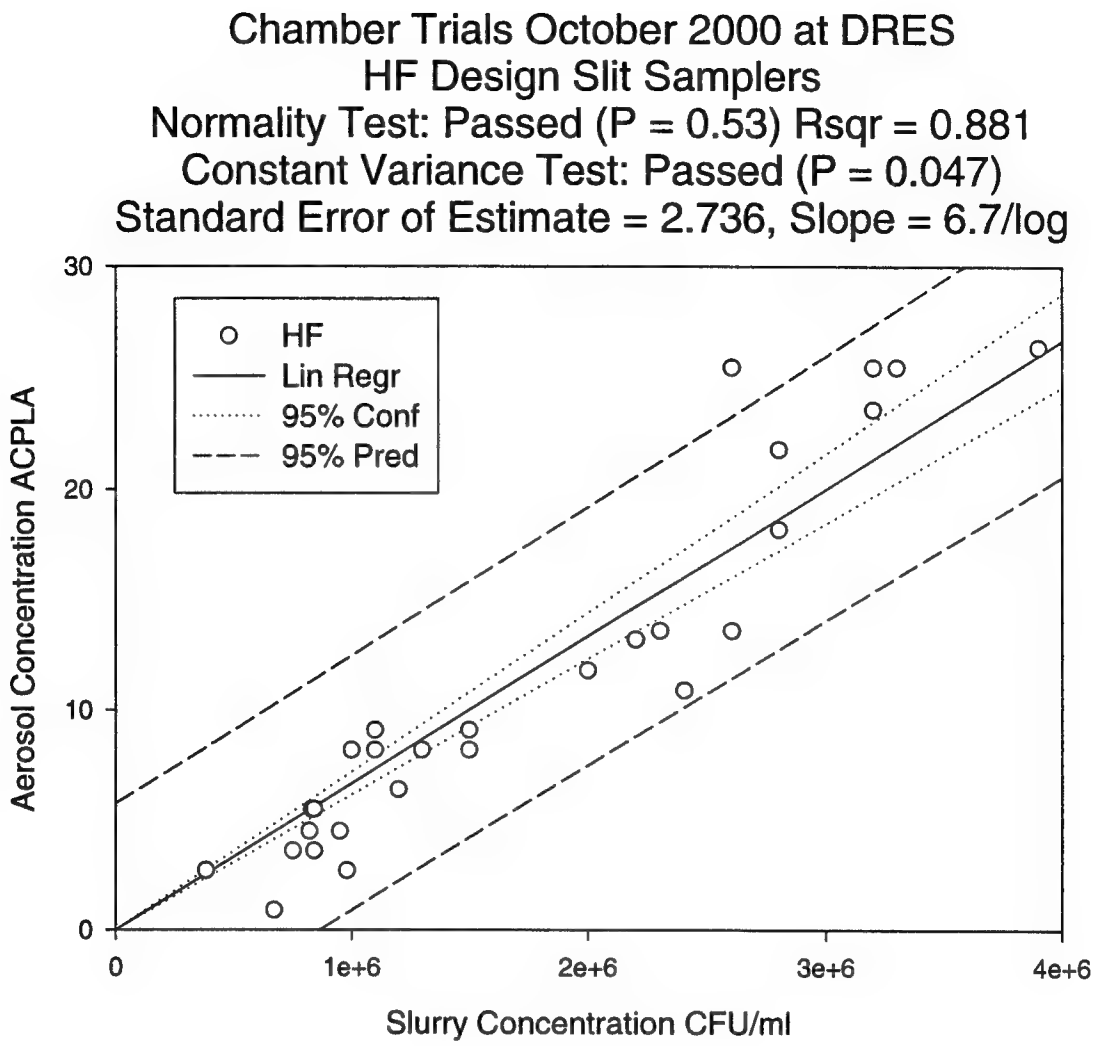


Figure 8. ***Plot profile of the HF modified samplers.***

What does regression slope mean

The experiments were designed with the expectation that an increase in spore suspension concentration in the generator should lead to a corresponding increase in viable aerosol particles in the chamber. A perfect slit sampler with 100% collection efficiency would give a straight line plot similar to the data in Figure 10 but its regression slope would have the greatest value. Due to indefinable factors like particle aggregation, wall losses and other physical interactions between aerosol particles, real samplers will have slopes that are less than this hypothetically perfect device. By the same rationale, the device with the best slope could be considered the best performer. There is a need to statistically compare the slopes derived from the various instruments and also to determine if there is one that is significantly better than the others. Regression slope values are summarized in table 2. By inspecting this

table, it would appear that the HF design gave the best slope value. Clearly there is a need for an objective way to determine if indeed this were true.

Table 2. Summary of statistical analysis

Slit Sampler	Normality P value	Variance P value	Standard Error of Estimate ACPLA	Regression coefficient R^2	Slope ACPLA per log slurry
Mattson Gavin	0.325	0.922	1.413	0.938	5.4
Dycor mod New Brunswick	0.670	0.632	2.075	0.853	4.2
DRES HF	0.532	0.047	2.736	0.881	6.7
10 cm design	0.462	0.268	0.588	0.918	2.2

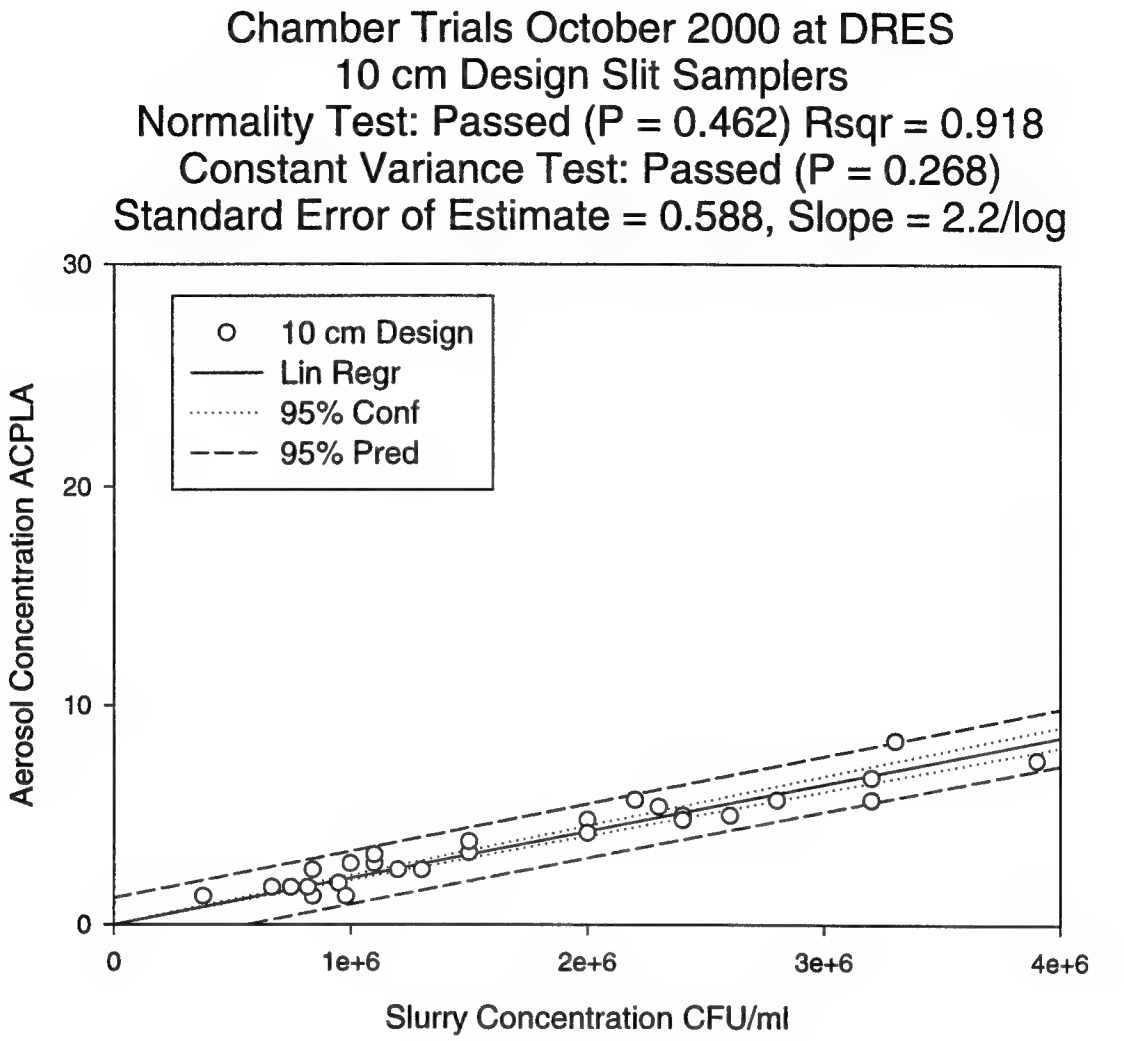


Figure 9. ***Plot profile of the 10 cm design samplers.***

Statistical methods to compare regression slope

Armitage and Berry (1995) described a method to compare two regression lines to determine if the slopes are significantly different. Zar (1998) extended this technique to include a way to compare more than two regression lines at the same time. In this report we applied Armitage and Berry's procedure because it was easier to implement as an Excel spreadsheet function and standard t distribution tables were available (Fisher and Yates 1963). The results of comparison between pairs of instruments are shown in table 3. Some conclusions can be drawn from inspecting this table; it shows that the HF slope is significantly different from that of the 10 cm design at 99.999% certainty. As these instruments registered the highest and the lowest slope values, we interpret the data as representing the two extremes of instrument performance. Similarly, the results in table 3 also

suggest that the HF design performed better than the Mattson-Gavin and the Dycor modified devices. There was no difference between the Mattson-Garvin and the Dycor modified systems.

Table 3. Comparison of slopes for the regression lines from various slit sampler designs. Values given represent significant difference at P taken from standard t distribution tables (Fisher and Yates 1963). For example, comparing the Dycor modified vs HF designs, a P value of 0.01 means there is a 99% probability that the slopes from each linear regression plot are significantly different. Note that each pair of regression data set was tested to derive a P value (Armitage and Berry, 1995)

	Mattson-Garvin	Dycor modified	HF	10 cm design
Mattson-Garvin		No Difference	0.02	0.001
Dycor modified	No Difference		0.01	0.001
HF	0.02	0.01		0.001
10 cm design	0.001	0.001	0.001	

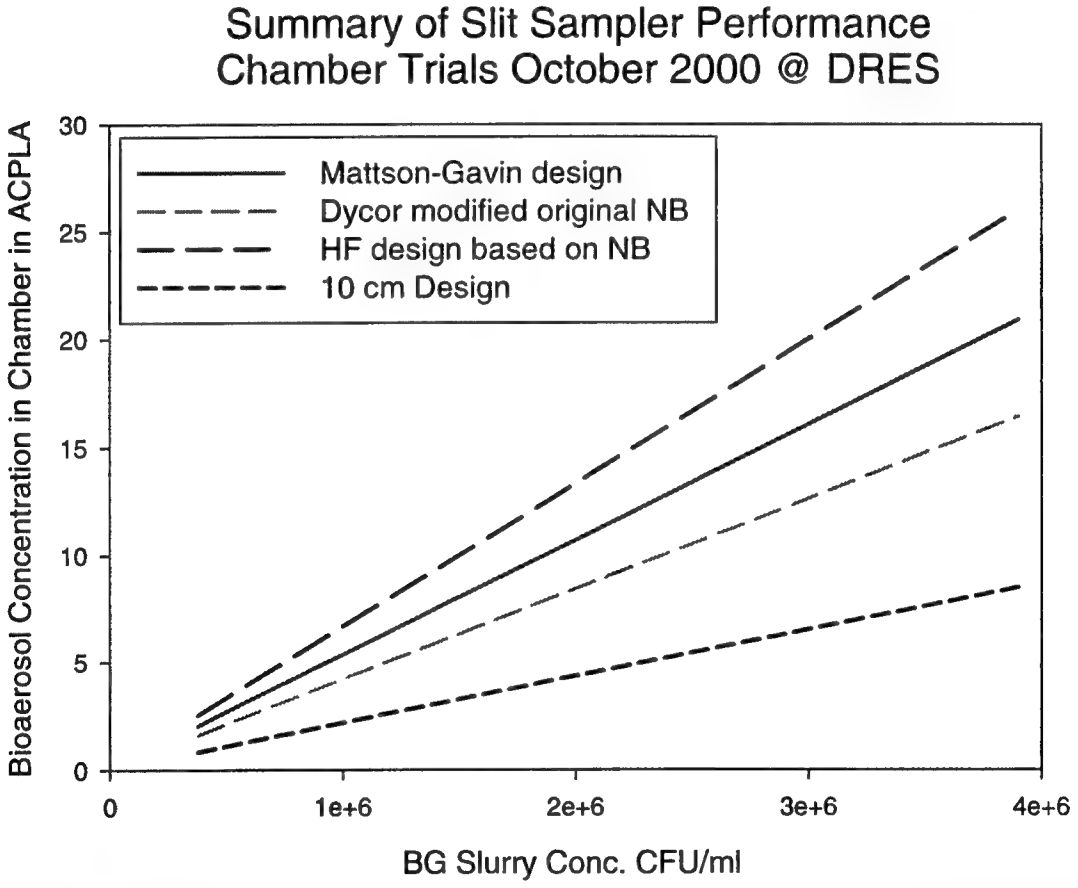


Figure 10. ***Summary of the linear regression results from all the samplers under test.***

However, it was noted in table 1 that the Dycor modified samplers were operating at below the rated flow rates but the ACPLA estimates were done using full flow values. Perhaps with the correct application of the measured flow rates for each instrument in the array, the performance characteristics could be different. We proceeded to apply the proper flow values to determine if its system performance would show up differently. It is interesting to note that when the Dycor modified sampler ACPLA results were recalculated using the correct flow rates from Table 1, the new slope obtain (Figure 11) was the same as that for the HF design (6.7). That the HF and the Dycor modified samplers have been demonstrated to perform similarly made perfect sense. We had used the same physical dimensions as well as the original slit and bowl for construction of these instruments.

When these samplers were tested under a different setting in another laboratory, it was confirmed that the 10 cm design was the least efficient (Burke et al 2001). Moreover,

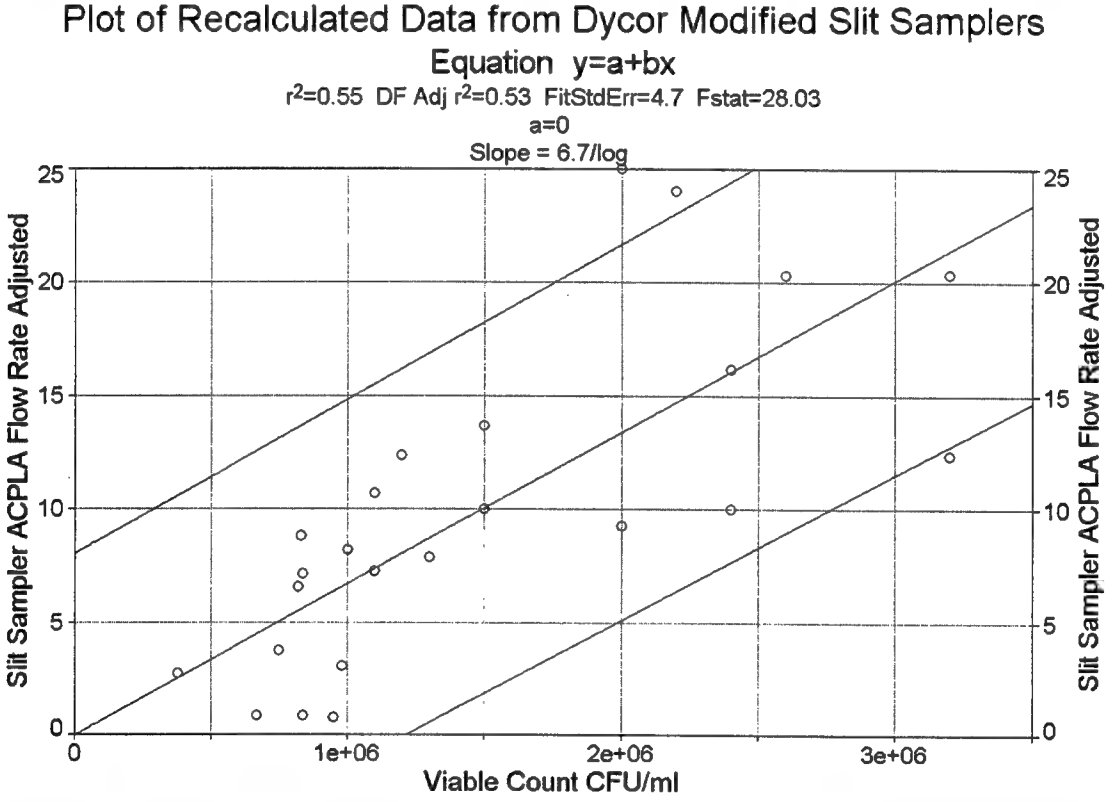


Figure 11. ***Replot of recalculated data for Dycor modified slit samplers. Flow rate values for each sampler indicated in Table 1 were applied to readjust the ACPLA results to give better representation of sampler performance. The linear regression line is shown with accompanying 90% prediction boundaries. Graph and statistics were generated by using TableCurve 2D fitting the data to a straight line equation.***

increasing the flow rate from 8 to 25 liter/min caused a decrease in performance. The authors explained that the low performance could be due to the smaller impingement surface, hence,

resulting in errors related to overlapping of impacting particles. Chang et al (1995) came to the same conclusion in their studies comparing the Mattson-Gavin versus the Casella samplers. They noted that the collection areas were 112.9 and 46.3 cm^2 respectively, making the Casella more susceptible to colony overlap errors. It is obvious that the 10 cm plate design is most suitable for clean air environments that have sparse viable particle populations.

Overlap error was most apparent when comparing low and high resolution samplers. This is shown in Figure 12 where *Erwinia herbicola* aerosol was disseminated and measured in the field as previously described (Ho et al 1999). In this figure, the high resolution samplers resolved sharp peaks when brief puffs of aerosol appeared. In contrast, the low resolution plot was represented as broad low yield curves. Most of the cloud events appeared to be coincident time-wise. However at some events, for example 14:22:30, 14:24:00 and 14:28:30 where extremely narrow puffs were present, the low resolution sampler hardly registered.

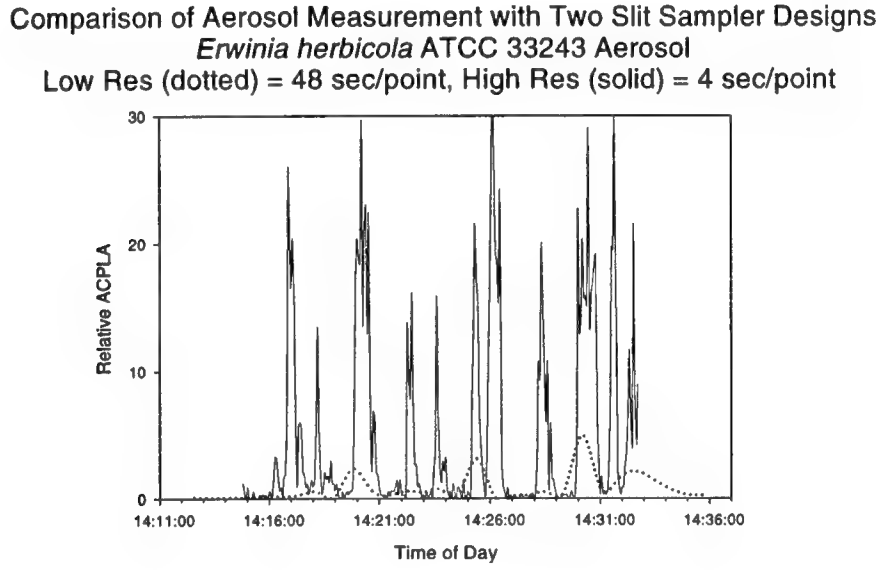


Figure 12. ***Measurement of a biological aerosol cloud using two kinds of slit sampler design. The low resolution instrument is a conventional New Brunswick sampler running at the standard 24 minutes per revolution. The high resolution instrument was running at 2 minutes per revolution. Both ran at similar flow rates.***

Most surprisingly, when they did register, the ACPLA levels were only about 20% of that from the high resolution equivalent (to be used later as a correction factor for low resolution instruments). This has very serious implications in that much of present and historical knowledge on biological aerosols have been obtained from interpreting observations with low resolution slit samplers. We have discovered that sampling environmental microbial

content in the tropics of northern Australia could easily overwhelm a low resolution slit sampler (http://www.dres.dnd.ca/cwal/Intro%20tindal99.htm). This became a major problem when some of the bacterial colonies contained "swarming" organisms that cause rapid spreading of boundaries making accurate counting difficult.

Towards deriving an instrument transfer function

The preceding discussions give the impression that the various slit samplers have definable performance characteristics and that each one can be summarized by its linear regression and slope (Figure 10). If indeed each instrument should actually follow the slope function throughout the range of aerosol concentration then by dividing the slope of one candidate instrument by that of the others, a transfer function is obtained. For example, using the HF and the 10 cm design to illustrate, and using the slopes from table 2 we get (6.7/2.2) 3.045 as the transfer function (TF). The true number is 3.065369 if rounding errors were eliminated. By multiplying any performance value from the 10 cm design by the TF, the resultant value would be equivalent to that obtained by the HF instrument. Again by inspecting Figure 10, taking the Y value at X= 2069600 we get 4.508. Multiply that by the TF we end up with 13.8186. This is the Y value for the HF sampler at the same X location.

Similarly TF can be derived for other instruments, thus permitting corrections to linear prediction of instrument performance under ideal conditions. Perhaps this might lead to a way to correct for the data obtained from historical slit samplers of less than optimal efficiency characteristics. Indeed, even for modern instruments like the Mattson-Garvin, a transfer function of 1.244139 can be used to estimate "true" ACPLA values. Although not strictly recommended, in a pinch, one could inspect Figure 13 to derive a slope value if only the slit length of a sampler is known. By this exercise, it may be possible to extract a transfer function to apply to correcting historical data. To compensate for low resolution characteristics, another correction factor of 5 should be included to take care of colony overlap errors. Thus the final formula for correcting for a short slit length sampler running at slow turntable speeds would take this form:

"True ACPLA" = measure ACPLA X transfer function X 5

As an application illustration we use the Casella slit sampler that has a slit length of 28 mm. By inspecting Figure 13, a theoretical slope factor 3.7 is obtained. This will give a transfer function of 1.8 (6.7/3.7). Assuming it has a low resolution turntable, a correction factor of 9.05 (1.8 X 5) is derived. In biological work, a correction factor of about 10 is a significant occurrence. It would appear that when reviewing biological aerosol literature concerning Casella data, a conversion factor of this magnitude may be called for. Future chamber trials should include a Casella sampler to verify this prediction. One caveat that must be stated is that the assumptions made above are for instruments of 0.15 mm slit width. Larger slit widths would require slight adjustments to take into account differences in inlet velocity.

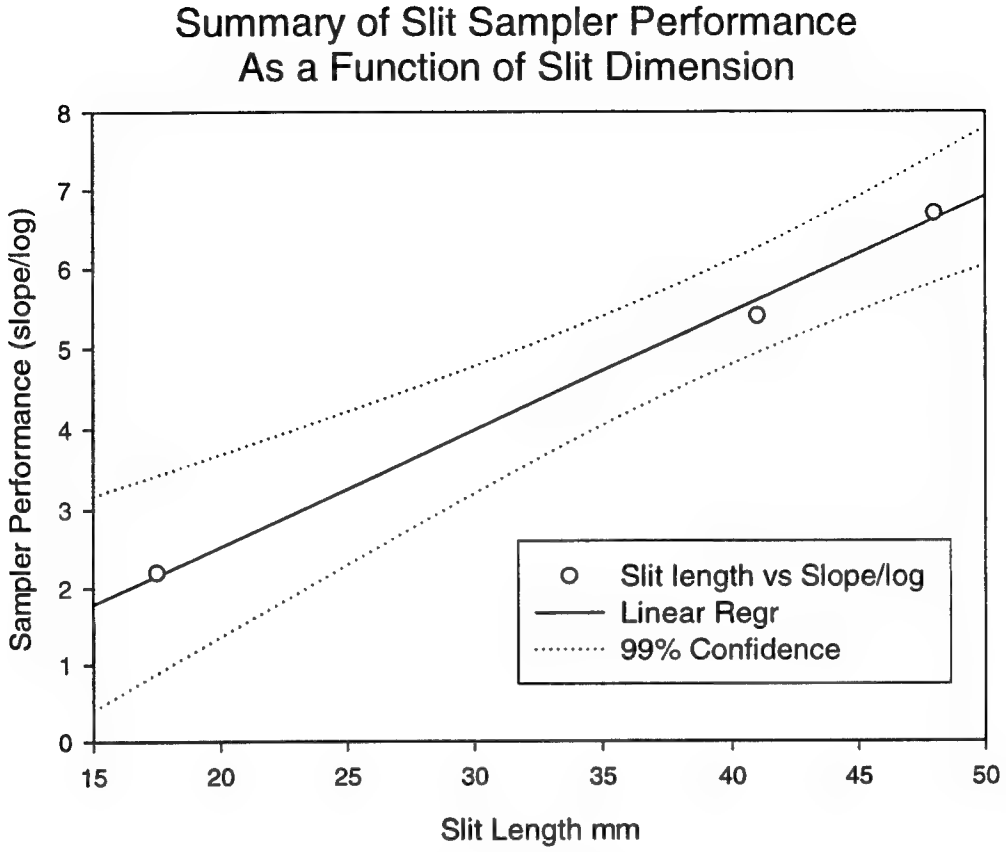


Figure 13. ***Relationship between slit length and sampler performance. The figure is derived from 4 data points with identical entries for the 48 mm instrument representing the two New Brunswick design modifications.***

Conclusion

We have examined the performance characteristics of different slit sampler designs, primarily ones with different slit length and impaction surface area. Having a short slit length design allows the use of smaller diameter petri plates for low cost particle collection. Although this may have economic advantages, there are definite compromises in terms of performances as depicted in Figure 13. Here it can be seen that there is a relationship between slit length and regression slope, the latter previously defined as a measure of sampling efficiency. We have also introduced a method to objectively compare regression slopes, providing an objective way to grade sampler performance. We recommend that future work with slit samplers should include verification that data collection has not been distorted by colony overlap errors. Previous workers (Upton et al 1995) have been concerned with sampling errors while using the slit sampler in horizontal laminar air flow like in a wind tunnel. Fortunately, in field application, horizontal laminar flow conditions were rarely encountered in nature. We respectfully submit that colony overlap errors may pose real problems when working in natural settings where biological content density can be difficult to predict. Finally, we have defined the concept of transfer functions as a way to correct for slit sampler design deficiencies, to be used for tweaking either historical or current data.

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The history on slit to agar samplers shows that the first published description was on a system using 10 cm petri plates. At some point after the 1950's samplers that collected biological aerosol particles on 15 cm plates appeared. More recent patents have been filed that described slit samplers using 10 cm plates, usually citing economics as the prime motivation. Yet workers have cautioned that the smaller plates can become saturated when heavy aerosol clouds are encountered. In applications where the slit sampler is used as reference collector when biological detectors are under test, heavy clouds may often be encountered. This report will demonstrate how to determine performance characteristics of slit samplers designed for either 10 or 15 cm plates. It will also show the importance of controlled replicate measurements providing data suitable for rigorous statistical analysis. The results indicate that for measuring biological clouds of between 5 to 30 agent containing particles per liter of air (ACPLA) the 15 cm plate sampler design is more efficient than those targeted for the smaller collection surface. A statistical method has been employed to test difference between regression slopes.

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